Targeting metastasis

Patricia S. Steeg

Abstract | Tumour metastasis, the movement of tumour cells from a primary site to progressively colonize distant organs, is a major contributor to the deaths of cancer patients. Therapeutic goals are the prevention of an initial metastasis in high-risk patients, shrinkage of established lesions and prevention of additional metastases in patients with limited disease. Instead of being autonomous, tumour cells engage in bidirectional interactions with metastatic microenvironments to alter antitumour immunity, the extracellular milieu, genomic stability, survival signalling, chemotherapeutic resistance and proliferative cycles. Can targeting of these interactions significantly improve patient outcomes? In this Review preclinical research, combination therapies and clinical trial designs are re-examined.

Standard of care
(SOC). Also known as best practice, treatment for each type and stage of cancer that is accepted in general practice by health care professionals, and iterated in guidelines such as those of the US National Comprehensive Cancer Network.

Metastatic colonization
The progressive growth of a lesion in a foreign location.

Invasion
Cancer cells traverse normal tissues in groups or as single cells, using reversible adhesion, proteolytic destruction and motility.

Genomic instability
A state of high frequency of mutations in a cell, including nucleic acid sequences, chromosomal rearrangements and aneuploidy.

Women's Malignancies Branch, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892, USA.
steegp@mail.nih.gov

Metastases, or the consequences of their treatment, are the greatest contributors to deaths from cancer. Clinical metastatic disease results from several selective forces. Pathways that fuel initial tumorigenesis, described as the 'trunk' of a cancer evolutionary tree, can also endow tumour cells with metastatic properties and *de novo* drug resistance. Two types of 'limb' pathway emerge from the tree trunk: events that induce acquired resistance to therapy and pathways that induce or accelerate metastasis to distant organs[1]. Cancer therapy has largely concentrated on druggable targets in the trunk tumorigenesis pathways, such as receptor tyrosine kinases, and uses sequential and combination therapies to minimize drug resistance. Metastasis-related limbs of the cancer evolutionary tree lag far behind in terms of identifying and drugging targets, validating their efficacy in rationally designed clinical trials and incorporating these therapies into the standard of care (SOC). Many of the metastasis-directed therapies under development are cytostatic, not cytotoxic, in preclinical experiments, making their clinical validation problematic. Overt scepticism exists in the pharmaceutical industry and some academic quarters about the concept of drugging metastasis. This Review challenges this notion with the hypothesis that our emerging understanding of metastasis, in particular the last step, metastatic colonization, will identify druggable pathways that will enhance the efficacy of current treatments.

What is metastasis? Genomically, analyses of matched sets of a patient's primary tumour and distant metastasis reveal mutations common to both and, almost universally, mutations that are distinct to a metastasis. Functionally, tumour cells begin metastasis by invasion of the tissue surrounding the primary tumour. Tumour cells enter the bloodstream, either directly or via the lymphatics system; traversal of the bloodstream most frequently

ends in arrest at the first capillary bed encountered. Tumour cells then extravasate the bloodstream to land on 'foreign soil'. Paget[2], 100 years ago, described metastasis in botanical terms as the interaction of 'seeds' (tumour cells) and 'congenial soil' (the metastatic microenvironment). How a foreign tissue becomes congenial contributes to metastatic colonization, that is, the progressive outgrowth of tumour cells at the distant site. The metastatic soil can be altered by bone marrow-derived cells before tumour cell arrival, termed the premetastatic niche. Eventually the cellular composition, immune status, blood supply, extracellular matrix (ECM) and virtually every other aspect of the metastatic site can be altered to favour colonization.

Besides the component pathways, other attributes of metastasis are important considerations in its therapeutic targeting. Multiple mechanistic pathways can mediate each of the requisite steps of metastasis. Like the repertoire of receptor tyrosine kinases, in which inhibition of one pathway can be overcome by activation of another kinase or a downstream mutation, inhibition of one metastasis pathway may be insufficient. Metastasis can pause part way — a state known as dormancy[3]. Do dormant metastatic cells require distinct therapeutic agents? Pathways mediating metastasis can be operative in multiple organs or they can be more site specific. Site-specific metastasis trials are becoming more common for bone and brain lesions. The metastatic process may begin early or late in primary tumour formation[4], and may require a brief period or decades to complete. These factors may influence patient selection and trial design. So when, in the patient's clinical course, do we most effectively halt metastasis? The fuel for metastasis may be genomic instability in all of its forms: metastases stand at the end of a progressive loss of the checks on normal chromosome stability, DNA repair and regulated
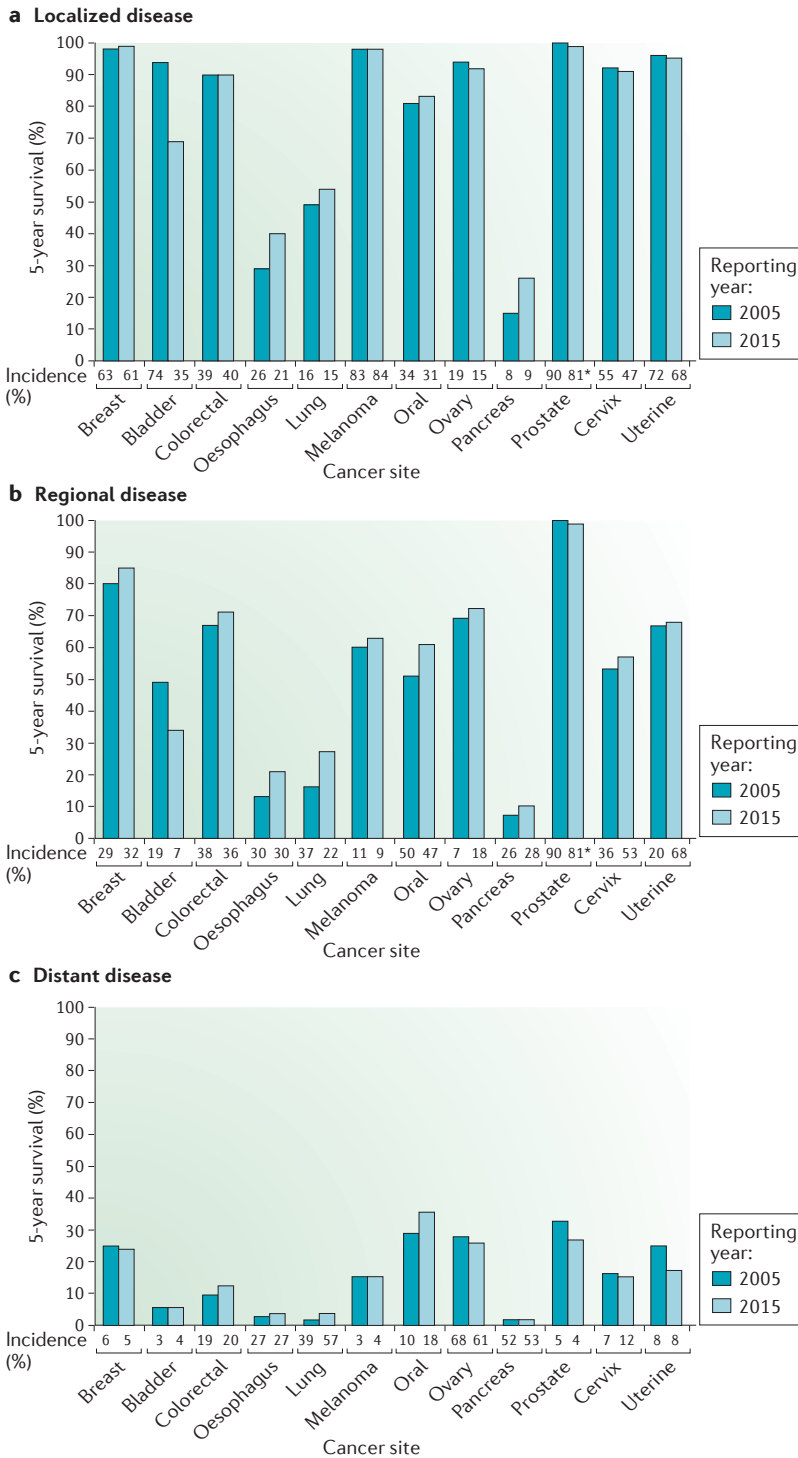
gene expression. Genomic instability can be found in a metastatically competent subclone of a primary tumour and/or can appear in the metastatic lesions[5–9]. Genomic instability is hypothesized to create many cellular phenotypes, any one of which may have all the necessary properties to complete the metastatic process. Can therapeutic targeting of processes that control genomic stability improve outcome? Few of the tumour cells that originally invade the surrounding tissue of the primary

tumour complete the metastatic process[10]; however, those that do go on to kill the patient. Can we identify the metastatically competent tumour cells or their products in the circulation as biomarkers or end points for earlier intervention? These and other complexities of metastasis must be thoughtfully confronted to produce successful drugs.

This Review identifies functional pathways of metastasis that are potentially efficacious for the prevention and treatment of metastases. It discusses the preclinical credentials that are required of lead antimetastatic agents. Finally, it looks into how we demonstrate an antimetastatic outcome in the clinic within reasonable time, patient and funding limits and how these drugs could be incorporated into the existing SOC.

Where are we?

Patient survival. For the overwhelming majority of cancer patients, a diagnosis of metastatic disease indicates a terminal illness. Although cancer death rates have declined, do patients with metastatic disease share equally in the improvements? Cancer incidence and 5-year survival data[11,12] provide a broad impression (FIG. 1). Patients initially diagnosed with localized disease often experience excellent 5-year survival (FIG. 1a). Those with regional disease at diagnosis (for example, patients with invasion of cancer to the regional lymph nodes) have lower survival overall, but, excluding patients with bladder or prostate cancer, patients often have survival gains between the 2005 and 2015 reporting periods (FIG. 1b). Only 4 of the 12 cancer sites assessed (colorectal, oesophageal, lung and oral) were associated with gains in the survival of patients with distant metastatic disease at diagnosis, and only 1 site demonstrated a survival gain of more than 3% (FIG. 1c). Alarmingly, the 5-year survival of several types of cancer (including ovarian, prostate and uterine cancer) decreased between the two reporting periods. These trends could be debated because newer immunotherapy and molecular

a Localized disease

Incidence (%) — Breast 63/61, Bladder 74/35, Colorectal 39/40, Oesophagus 26/21, Lung 16/15, Melanoma 83/84, Oral 34/31, Ovary 19/15, Pancreas 8/9, Prostate 90/81*, Cervix 55/47, Uterine 72/68

b Regional disease

Incidence (%) — Breast 29/32, Bladder 19/7, Colorectal 38/36, Oesophagus 30/30, Lung 37/22, Melanoma 11/9, Oral 50/47, Ovary 7/18, Pancreas 26/28, Prostate 90/81*, Cervix 36/53, Uterine 20/68

c Distant disease

Incidence (%) — Breast 6/5, Bladder 3/4, Colorectal 19/20, Oesophagus 27/27, Lung 39/57, Melanoma 3/4, Oral 10/18, Ovary 68/61, Pancreas 52/53, Prostate 5/4, Cervix 7/12, Uterine 8/8

Figure 1 | **Few improvements** in 5-year survival for cancer patients initially diagnosed with metastatic disease. The percentage of patients surviving for 5 years is plotted based on their initial disease staging of localized (organ confined), regional (invasion to lymph nodes) or distant (metastases detected by imaging) using the US National Cancer Institute Surveillance, Epidemiology and End Results (SEER) registries[11,12]. Data covering 1995–2000 and 2004–2010 were reported in 2005 and 2015, respectively, to determine where improvements were attained. With few exceptions, 5-year survival after a diagnosis of localized disease was excellent; where it was low in 2005, gains were observed in 2015. Regional disease survival rates fluctuated by cancer type, but the majority saw increased survival in the later reporting period. Patients with metastatic disease at diagnosis had lower overall 5-year survival rates, with fewer than 20% of patients surviving after 5 years for half of the cancer sites. The increase in survival between the 2005 and 2015 reporting periods was under 3% in three of the four cancer types for which increased survival was seen. For each type, stage categories may not total 100% because of insufficient information for all cases. Beneath each plot is the incidence of each stage at diagnosis for the reporting period. *Localized and regional data were combined.

advances were not incorporated. Recent immunotherapies have extended survival in melanoma[13], and new androgen receptor inhibitors have improved the survival of patients with metastatic prostate cancer[14]. However, independent analyses of survival in the metastatic setting for specific cancers paint a similarly dismal picture. Over a 30-year period, results of randomized clinical trials failed to show sustained evidence of increased survival of patients with metastatic breast cancer[15]. Modest gain or no gain in survival was reported for metastatic gastric and pancreatic cancers[16,17]. These reports also include data for patients who were diagnosed with non-metastatic disease and then became metastatic, in contrast to the more limited data from the Surveillance, Epidemiology and End Results (SEER) programme of the US National Cancer Institute. Current approaches to metastatic disease are not improving satisfactorily.

Metastasis as an uninvited aspect of traditional drug development. Most of the cancer drugs approved by the US Food and Drug Administration (FDA) or other regulatory agencies were preclinically validated as anti-tumorigenic and were initially tested in clinical trials that enrolled patients with metastatic disease. These trials recruit patients with measurable metastatic disease and ask whether a treatment will shrink established lesions (responses) or extend patient overall survival (OS) or progression-free survival (PFS). Success in the metastatic setting often sets in motion the next hypothesis: that the drug will be effective in preventing metastasis. Many of these drugs were not initially tested in metastatic preclinical models. It was assumed either that drugs that target tumour growth would also target metastasis, or that interrupting distinct metastatic limbs of the cancer evolutionary tree would not be necessary in the face of overwhelming growth inhibition. In the setting of adjuvant trials, patients with evidence of aggressive disease but no identifiable distant metastases are treated to prevent metastatic colonization, with disease recurrence, OS and PFS as end points. These trials assume that cancer is a systemic disease. The adverse effect profile is very important, as the patient population is healthier than patients with metastatic disease.

Where metastasis preclinical data have been reported in the traditional drug development process, they have often muddied the waters. Different androgen deprivation therapies exerted disparate effects on invasion and metastasis in prostate cancer by engaging distinct metastasis-associated signalling pathways[18]. Several approved drugs, including mutant BRAF inhibitors[19], paclitaxel[20], cisplatin[21], anti-androgens[22], everolimus[23] and sunitinib[24], have stimulated metastasis in preclinical models. These stimulatory effects may result from systemic toxic effects, initiating wound healing-type recovery that is laced with growth factors used by metastasizing tumour cells[25]. Preclinical metastasis data are often directed at identifying resistance mechanisms and potential rational treatment combinations[26,27].

Although progress in the adjuvant setting, and to a lesser extent the metastatic setting, is undeniable, would the identification of therapies that halt metastasis improve

outcomes for patients? Would we select different lead agents or combinations by incorporating preclinical data on metastatic progression?

A metastasis drug development report card. Given the potential contributions of metastasis to drug development and patient outcomes, how have the several recent attempts to incorporate metastasis pathways and end points into drug development fared? TABLE 1 summarizes the preclinical and clinical experience for four potential antimetastatic drugs. Denosumab, a humanized monoclonal antibody that binds receptor activator of NF-κB ligand (RANKL; also known as TNFSF11), interrupts the 'vicious cycle' of bone metastasis colonization. In the vicious cycle, tumour cells arriving in the bone produce factors that activate bone-forming osteoblasts to produce RANKL, which in turn activates bone-destroying osteoclasts to degrade bone. As it is destroyed, the bone matrix releases bound factors such as transforming growth factor-β (TGFβ), activating tumour cells and reinitiating the cycle[28]. Preclinical data demonstrated that denosumab hit its intended target in healthy mice[29]. In the metastatic setting, trials of denosumab enrolled patients with bone metastases and used an unusual metastasis-relevant end point, a skeletal-related event (SRE). This is a deleterious event such as a bone fracture from expansion of an existing metastasis or a new metastasis. Significant reductions in SREs for denosumab compared with SOC were observed for both breast and prostate cancer[30,31]. No difference was observed in the traditional OS end point. Denosumab was then tested in adjuvant trials and was shown to delay initial bone metastases in patients with castration-resistant prostate cancer or patients with postmenopausal breast cancer on aromatase-inhibitor therapy[32,33].

The responses to bevacizumab, a humanized antibody to vascular endothelial growth factor (VEGF) — which is important in angiogenesis — have been mixed. Angiogenesis is hypothesized to contribute to metastatic colonization by providing new capillaries to deliver oxygen and nutrients[34]. VEGF is a growth and permeability factor for capillary endothelial cells. Preclinically, bevacizumab was initially tested on corneal angiogenesis, multiple primary tumours and, occasionally, metastasis models. Trials were conducted in the initial or refractory (after progression on other therapy) metastatic settings with survival end points. Bevacizumab is approved by the FDA for several cancers (FDA approval for bevacizumab), although the absolute increases in survival have been nominal in ovarian[35,36] and refractory non-small-cell lung cancer[37]. Conditional approval was revoked in breast cancer[38] and other cancers were negative for survival end points[39,40]. Adjuvant trials were negative in triple-negative breast cancer[41] and colorectal cancer[42,43]. The lack of adjuvant efficacy in breast cancer contrasts with an increase in pathological complete response (pCR), or the complete disappearance of a primary tumour, in a neoadjuvant trial (treatment before surgery) using a similar patient population[44,45].

What is behind these mixed results? Preclinical data indicate that bevacizumab can initiate a vascular remodelling response, to normalize vessels and render them

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Overall survival
(OS). The length of time, either from disease diagnosis or the beginning of treatment, until death.

Progression-free survival
(PFS). A metric of patient response to therapy, measured from the time of treatment initiation or clinical trial enrolment until either detectable lesions increase, based on standard measurement criteria, or the patient dies.

Adjuvant trials
Clinical trials to test whether an additional treatment after primary therapy will lower the risk of cancer recurrence.

Pathological complete response
(pCR). The absence of residual invasive tumour cells by microscopic examination of resected tissue after neoadjuvant therapy.

Neoadjuvant trial
In cancer, a trial testing a potential therapy before the 'definitive' treatment, such as primary tumour surgery.

resistant to the drug[46]; bevacizumab also stimulates compensatory pathways, increasing tumour cell motility and invasion[47,48]. Other anti-angiogenic regimens shrink primary tumours but enhance metastasis in response to the resultant hypoxia[24,49–51]. Bevacizumab seems to have been a good prospect for targeting tumours but was it an optimal one for targeting metastases? Additional contributing factors include the existence of other functional pathways that regulate angiogenesis, for instance, the angiopoietin 2 (ANGPT2) pathway involved in vessel stabilization[52]. It will be of interest to determine whether targeting multiple aspects of angiogenesis provides a

Table 1 | Preclinical and clinical history of four metastasis-directed drug development efforts

Description	Pathway	Preclinical validation	Pivotal trials and end points	Outcomes
Denosumab				
Monoclonal antibody to RANKL		RANKL activates osteoclasts and promotes bone destruction; denosumab reduced bone resorption in mice expressing human RANKL[29]	SREs* in metastatic setting; adjuvant trials used time to first bone metastasis or fracture[30–33]	FDA approved for prevention of SREs in solid tumours; approved as adjuvant therapy in prostate cancer
Bevacizumab				
Monoclonal antibody to VEGF		• Bevacizumab inhibited corneal angiogenesis and lymphangiogenesis[244] • In multiple cancer xenograft models, bevacizumab reduced primary tumour growth rates and, in some studies, enhanced survival. Reduced angiogenesis and vessel normalization was observed[245] • Prevention or, less frequently, abrogation of metastasis[246,247]	• Recurrent ovarian cancer, PFS[35,36] • Metastatic colorectal cancer, OS[260,261] • Metastatic or resistant HER2+ breast cancer, PFS[38] • Metastatic renal cancer, PFS[262] • Glioblastoma, OS, PFS[263] • Advanced lung cancer, OS[37] • Adjuvant therapy in triple-negative breast cancer, DFS[41]	• FDA approved for resistant ovarian, cervical and colorectal cancers, glioblastoma, also advanced or metastatic lung, colorectal and renal cancers • Revoked for metastatic breast cancer • Negative trials for first-line treatment of glioblastoma
Cilengitide				
αvβ3 and αvβ5 integrin peptide inhibitor		• Stabilization of glioma growth and angiogenesis. Synergistic inhibition of glioma with TMZ[61–64] • Synergy with therapeutics in melanoma primary tumour growth[63], synergy with radio-immunotherapy in breast cancer tumour growth[248] • Inhibition of metastasis[62] • Synergy with verapamil increased angiogenesis and reduced metastasis[249]	• Phase III CENTRIC EORTC, with radiation therapy and TMZ, for glioma, OS. Newly diagnosed glioma, same combination, recurrence[65] • Phase II trials in melanoma and lung and prostate cancers, PFS[66–68]	All advanced trials were negative
Dasatinib and saracatenib				
SRC kinase and BCR–ABL kinase inhibitor		• Inhibition of CML models[250] • Inhibition of primary tumour growth in multiple model systems, as monotherapy or in combination[251–253] • Prevention of metastasis in multiple cancer model systems[254–258], but not osteosarcoma[259] • Inhibition of prostate cancer growing in bone and bone remodelling[82,83]	• Cytogenetic response end points for CML • Response for advanced solid tumours[71–80] • OS in Phase III prostate cancer[87]	• FDA approved for CML and resistant ALL • Discontinued in advanced lung, ovarian, colorectal and breast cancers • Negative in prostate cancer Phase III trial with docetaxel • Multiple adjuvant trials terminated

ALL, acute lymphoblastic leukaemia; CML, chronic myelogenous leukaemia; DFS, disease-free survival; ECM, extracellular matrix; FAK, focal adhesion kinase; FDA, US Food and Drug Administration; OS, overall survival; PFS, progression-free survival; RANK, receptor activator of NF-κB; RANKL, RANK ligand; RTK, receptor tyrosine kinase; TMZ, temozolomide; VEGF, vascular endothelial growth factor. *Skeletal-related event (SRE) captures the deleterious effects of new lesions and progression of existing lesions to cause patient morbidity.

more substantial survival advantage. Also, the requirement for angiogenesis in metastasis varies[53,54]. In some models angiogenesis is unnecessary — co-option of the existing vasculature is sufficient for metastasis, or tumour cells induce a vascular network[55–57].

Cilengitide, a cyclic peptide inhibitor of αvβ3 and αvβ5 integrins, has been considered as a prospective candidate for metastasis therapeutics. Integrins are receptors that consist of one each of several possible α and β subunits and they mediate the adhesion of tumour cells to the ECM to affect angiogenesis, viability, invasion and colonization[58–60]. Preclinical work demonstrated prevention of metastasis by cilengitide as monotherapy[61,62] or in combination with other agents[63], as well as prevention of glioma growth and invasion[64]. Clinical development proceeded through Phase III trials in glioma and were negative for an OS end point[65]. As glioma progression does not involve distant metastasis, these trials could potentially be dismissed. However, Phase II trials were conducted in patients with bone metastatic prostate cancer, metastatic melanoma and advanced non-small-cell lung cancer without showing significant clinical activity[66–68]. Failure here may be due to simple drug development principles rather than an absence of a role of these integrins in the metastasis pathway, as the compound had a very short half-life *in vivo*.

Another set of disappointments were the SRC inhibitors dasatinib and saracatinib. Dasatinib inhibits the BCR–ABL fusion protein underlying chronic myelogenous leukaemia (CML) and the SRC non-receptor kinase. Saracatinib is an independent inhibitor of the same targets. SRC is phosphorylated downstream of multiple receptors, including those for adhesion and cytokines, as well as receptor tyrosine kinases and G-protein-coupled receptors. SRC signalling is best described in tumour motility and invasion, in which activated SRC forms a complex with focal adhesion kinase (FAK, also known as PTK2), forming focal adhesions, lamellipodia and stress fibres, and causing contraction of the actin cytoskeleton; roles in angiogenesis, proliferation and survival are also documented. SRC activation stimulated metastasis in multiple model systems[69]. Metastasis was significantly prevented when dasatinib or saracatinib was given early and continuously in model systems, including pancreatic, thyroid, prostate, urothelial, ovarian and gastric cancers, melanoma, multiple myeloma and fibrosarcoma, and synergized with several other drugs. Regression of lesions was infrequently demonstrated. In breast cancer, primary tumours regressed using a dasatinib combination with rapamycin, an mTOR inhibitor. In this same model, dasatinib reduced the number of lung metastases, but no additive or synergistic effect was observed with the dasatinib plus rapamycin combination[70].

On the basis of this substantial preclinical evidence, SRC inhibition was anticipated to be a blockbuster antimetastatic agent. Dasatinib is approved by the FDA for treatment of CML and relapsed acute lymphoblastic leukaemia based on its BCR–ABL kinase inhibitory activity (FDA approval for dasatinib), but its clinical activity in metastatic disease has been nothing but disastrous. Saracatinib development was discontinued by its manufacturer. The overwhelming majority of the trials conducted were in the metastatic setting with response and PFS as end points, and both drugs sometimes resulted in long-term stable disease. As monotherapy, saracatinib and dasatinib were both negative in trials of hormone receptor-negative breast cancer[71,72], hormone receptor-positive or HER2 (also known as ERBB2)-positive advanced breast cancer[73], recurrent or persistent ovarian cancer[74], refractory colorectal cancer[75], advanced melanoma[76,77], extensive stage small-cell lung cancer[78], recurrent or metastatic head and neck cancer[79] and metastatic or locally advanced gastric cancer[80]. Combination trials in similar settings were negative, as was a trial using a gene signature of SRC activation to personalize trial enrolment[81]. Bone metastasis in castration-resistant prostate cancer provides another example: in mice, saracatinib in combination with docetaxel inhibited bone turnover, prevented bone metastasis[82] and inhibited growth of tumour implanted into bone[83]. In patients with refractory disease, dasatinib monotherapy was negative for a response end point[84]; when administered to chemotherapy-naive men, dasatinib produced stable disease and reductions in bone turnover markers in urine[85,86]. The combination of dasatinib and docetaxel was negative for an OS end point[87].

These data actually raise a wealth of potential reasons why SRC inhibition may still be a good antimetastatic agent. First, the overwhelming majority of the preclinical data indicated a prevention of metastasis, not a shrinkage of overt lesions. This would be tested in an adjuvant trial. The trial end points may be wrong: for the prostate cancer bone metastasis trials, the SRE end point that was successfully applied to the denosumab trials was not used. The patient populations used may have been inappropriate for the trial: the role of SRC inhibition in chemoresistant disease was not established preclinically, but this patient population was frequently enrolled; the prostate cancer trials using earlier, chemotherapy-naive patients were the most promising. Second, drug combination studies in general may be problematic. Preclinical studies are often conducted using a low dose of both drugs to see a statistical interaction. In the clinic, both drugs are used at or near the maximum tolerated dose (MTD). Is this the same? Third, standard drug development features of the SRC inhibitors may have been important; substantial Grade 3 and some Grade 4 adverse reactions occurred. Would the side effect profile preclude an adjuvant trial in healthier patients?

In summary, denosumab indicates that metastasis can be drugged. Attributes of this effort included preclinical experiments conducted in the target organ of metastasis and a clinical trial design based on a relevant end point caused directly by the metastatic pathway. Limitations identified in other drug development efforts include an inadequate understanding of the molecular pathway in metastatic colonization, poor drug characteristics, overinterpretation of early-phase trial data, a preclinical focus on effects on the primary tumour and the wrong trial design.

Half-life
In pharmacology, the time it takes for a compound to fall to one-half of its initial steady-state level.

G-protein-coupled receptors
A family of integral membrane receptors that sense extracellular signals and activate intracellular signalling by binding to G proteins.

Focal adhesion kinase
(FAK). Cytosolic non-receptor protein kinase typically linking extracellular matrix with the actin network, regulating cell adhesion, viability and spreading.

Stable disease
A metric of patient response to therapy, in which measurable lesions are neither increasing nor decreasing based on standard measurement criteria.

Maximum tolerated dose
(MTD). The highest dose of a drug or treatment that does not cause unacceptable side effects.

REVIEWS

New targets

Which part of the metastatic cascade? Many pathways have been validated to facilitate or interrupt metastasis, but have yet to be drugged. The most promising candidate pathways will not only be functionally validated but will be open to intervention in many patients after diagnosis.

The entire metastatic process represents a potential therapeutic target for patients with localized disease. However, localized disease represents a minority of certain cancers at diagnosis (localized disease is particularly rare for patients with cancer of the ovary, pancreas, oral cavity, lung, oesophagus and colorectum; FIG. 1) and is therefore of limited applicability. For regional disease, the likelihood of distant metastasis formation increases, as reflected in survival rates. Regional disease includes more than a quarter of all diagnosed breast, colorectal, lung, pancreatic, cervical, oral and oesophageal cancers (FIG. 1b). At this stage, tumour cells have probably spread systemically and are sitting dormant in distant organs or beginning to colonize, but are too small to be detected by imaging. Adjuvant systemic therapy is administered but unfortunately does not adequately control progression. What remains as an open therapeutic window is the metastatic colonization process. Could interruption of mechanistic pathways mediating metastatic colonization supplement standard adjuvant regimens to prevent further progression and improve survival? For patients with limited, treatable metastatic disease, interruption of the metastatic colonization by other tumour cells in the distant organs could prevent the outgrowth of additional metastases.

Seeding. Many mechanistic pathways are involved in the initial invasion of tumour cells from the primary site: travel through the circulation, arrest either at the next capillary bed or in a site-specific manner, and extravasation. These include: tumour–tumour and tumour–ECM adhesion molecules; diverse proteases; plasticity programmes such as the epithelial–mesenchymal transition (EMT) and stem or tumour-initiating cell pathways that are fuelled by EMT; anoikis; and adhesion to the vascular endothelium. As noted above, most cancer patients at the time of diagnosis may have already completed these processes, leaving them unavailable for therapeutic intervention. An exception would be the reseeding of metastases from established metastases. Clinical evidence for this is emerging from DNA sequencing studies[88], but additional human and preclinical mechanistic data are needed to support translational efforts.

Dormancy. For breast and prostate cancers, metastatic colonization is delayed by years or decades in a proportion of patients, a process termed dormancy. Dormancy may be a key therapeutic window by which to target metastatic colonization. Clinically, dormancy is defined as an unusually long time between removal of the primary tumour and subsequent relapse in a patient who has been clinically disease free. In preclinical models, tumour cells disseminate but do not steadily form overt metastases. Tumour cells can enter dormancy nestled

in their eventual metastatic site; alternatively, tumour cells are found in bone marrow, with prognostic relevance[89]. Bone marrow may constitute a reservoir for dormant tumour cells that can eventually mobilize and colonize elsewhere.

Dormancy can be achieved by many means — for example, an exit from the cell cycle by tumour cells, balanced proliferation and apoptosis signalling, or host responses such as angiogenesis or immune activation. In immune dormancy, immunoediting may occur, whereby tumour cells expressing strong neoantigens are eliminated by the immune system. Dormant residual tumour cells expressing relatively weak antigens remain, to escape if further evolution blunts immune control[90]. In this scenario, it could be hypothesized that dormant tumour cells expressing relatively weak antigens may be refractory to immunotherapies upon relapse.

A lack of preclinical model systems that address the complexity of dormancy has precluded widespread research. In the past, many poorly metastatic cell lines were established and compared with related, more aggressive cell lines[91]. With more modern testing, it is possible that they may provide additional dormancy model systems. Even with a model in hand, experiments require relatively long times and the end points are difficult, that is, identification and characterization of single tumour cells in distant sites. Areas of research interest include the identification of niches that promote dormancy[92,93], dormancy as a p38 (also known as MAPK14)-driven stress response[94,95], the relationship of stem cell pathways and dormancy[96], tumour cell adhesion molecules[97] and ECM cues for reactivation of cell growth[98].

To illustrate the potential clinical relevance of cell cycle exit in dormancy, a hormone receptor-negative breast cancer preclinical model was used. Mice were injected with either a steadily metastasizing or a dormant cell line; they were randomized to vehicle or the chemotherapeutic agent doxorubicin. The progressing cell line produced abundant metastases, which were reduced by drug treatment. The dormant cell line produced few metastases, which were unaffected by the drug[99]. The dormant tumour cells were insensitive to traditional antiproliferative drugs.

Potential translational approaches to dormancy include targeting signalling pathways that maintain the dormant state[95], synthetic lethal combinations to kill G0 tumour cells, monoclonal antibody targeting of single tumour cells and extension of the length of maintenance anti-hormonal treatments[100]. As an example, a small-molecule inhibitor of the tumour cell lysophosphatidic acid receptor 1 (LPAR1) pathway not only prevented overt metastasis formation in models of breast cancer, but shifted the majority of the remaining disseminated tumour cells to the G0 resting state and activated p38 stress signalling[95]. This type of inhibitor may stand as a candidate dormancy-inducing agent. One of the simplest ways to test this hypothesis clinically may be to enroll patients with breast cancer who are at highest risk of metastasis, such as patients with remaining primary tumours after neoadjuvant chemotherapy and surgery, patients with multiple positive lymph nodes or patients

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Extravasation
In metastasis, the movement of tumour cells out of the circulatory system into surrounding tissues.

Stem or tumour-initiating cell
A cell found within a cancer that is tumorigenic and can differentiate into one of several cell types found within the tumour.

Anoikis
A form of programmed cell death induced by anchorage-dependent cells detaching from an extracellular matrix.

Neoantigens
Peptides absent from the normal genome, caused by somatic mutations.

Myofibroblasts
Cells with attributes of fibroblasts and smooth muscle cells that are activated to participate in wound repair.

Mismatch repair
A form of DNA repair that corrects erroneous misincorporation of bases during replication, and other insertions, deletions and DNA damage.

Double strand break repair
Repair of hazardous lesions in which both strands of DNA are broken, by non-homologous end joining or homologous recombination repair.

with chest wall recurrences. After randomization to the intervention or placebo, the primary end point would then be time to the development of a distant metastasis. Further validation of potential biomarkers of residual disease, such as circulating mutant DNA may provide a secondary readout.

Metastatic colonization. Metastatic colonization remains the optimal window for therapeutic development in prevention of metastasis in the adjuvant setting, and prevention of the development of additional lesions in the limited metastatic setting. So, what is metastatic colonization, and why is it not just like primary tumour growth? Metastatic colonization fuses pathways and alterations found in the primary mass, early events in metastasis and events important to outgrowth in a foreign location. Genomic alterations provide examples: each tumour has oncogenic mutations; they represent the trunk of the cancer evolutionary tree. The prevalence and expression patterns of these mutations, for instance, in *TP53* (REF. 101), *KRAS*[101], *ESR1* (which encodes oestrogen receptor-α; ERα)[102,103], *MYC*[104] and *BRAF*[105,106],

may be further exacerbated in metastases. Many of the pathways affected by these truncal mutations have been demonstrated to affect metastatic ability[107–111], although a causal role in post-extravasation colonization has not been established.

In other cases, new genetic alterations, not seen in the matched primary tumour, are observed. These alterations are considered limbs on the phylogenetic tree, as they occur late in the evolution of the disease. In a landmark study of prostate cancer progression, amplification or mutation of both mismatch repair and DNA double strand break repair genes was a hallmark only of the metastases[9]. The data suggest that dual inhibition of DNA repair pathways, if achievable without synergistic toxicities, may be lethal to metastases. In a study of matched primary tumours and brain metastases, 53% of the brain metastases harboured clinically actionable mutations not seen in the patient's primary tumour[112]. These data suggest that therapies directed at truncal mutations may be efficacious in distinct clinical settings based on expression patterns in metastasis. Epigenetic changes are likely to outnumber genetic alterations and may change during the colonization process.
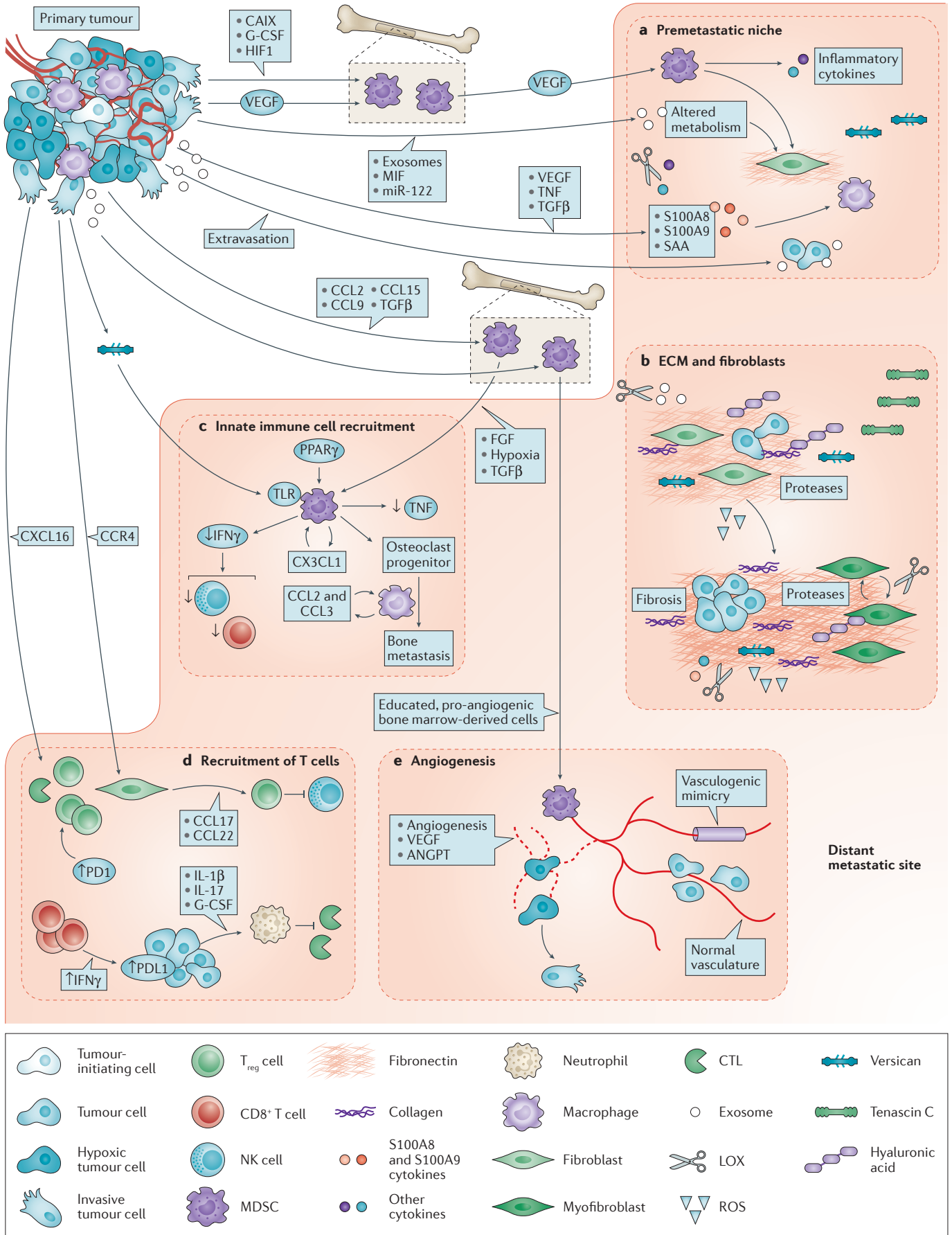
Metastatic colonization also mechanistically involves tumour signalling pathways regulating diverse cellular functions (BOX 1). In addition to these cell-intrinsic pathways, metastatic colonization represents the interaction between the tumour cell and its foreign microenvironment. FIGURE 2 outlines interactions between tumour cells and aspects of their new environment, including the premetastatic niche, the ECM, nonspecific immunity (also known as innate immunity), adaptive immunity, and angiogenesis. In general, tumour cells do not land in a foreign site and colonize it as it is; instead, they extensively modify the environment, recruiting bone marrow-derived cells and immune cells and activating wound response programmes in the tissue. Of the metastasis translational targets currently under development, TGFβ, a secreted cytokine, provides an example of the potential and possible pitfalls in targeting colonization (BOX 2). Each of these pathways may hold added attraction as potential therapeutic targets, in that inhibition of one protein often inhibits myriad downstream interactions and phenotypes.

Chemokines. A family of diverse chemokines regulates immune cell migration in inflammation and homeostasis by interacting with G-protein-coupled receptors (FIG. 2c,d). Chemokines such as chemokine (C-X-C motif) ligand 12 (CXCL12), interacting with chemokine (C-X-C motif) receptor 4 (CXCR4), are widely expressed and associated with multi-organ metastasis; other chemokines have been proposed to mediate organ-specific colonization, such as chemokine (C-C motif) receptor 9 (CCR9) stimulation of melanoma metastasis to the intestine[113]. Chemokines contribute to metastatic colonization by promoting the infiltration and survival of macrophages[114,115] and regulation of T cell-mediated antitumour immune responses[116]. Other chemokines are inhibitory. CXCL16 expression by colon cancer cells inhibited metastasis by attracting natural killer (NK) cells and CD8+ T cells that express CXCR6 (REF. 117).

Box 1 | Potential tumour cell targets in metastatic colonization

In addition to mutational events, tumour cells alter multiple signalling pathways in order to colonize a foreign organ. Many of the functionally validated signalling pathways in metastatic colonization focus on end points other than proliferation.

- Metastatic colonization involves the maintenance or enhancement of signalling pathways mediating tumour cell viability and resistance to death. Protection from apoptosis in metastatic colonization is afforded by autocrine interleukin-6 (IL-6)–signal transducer and activator of transcription 3 (STAT3) signalling, which reduces caspase 3 activation[162], overexpression of the CUB domain-containing protein 1 (CDCP1)-mediated anti-apoptotic pathway[163] and overexpression of the B-cell lymphoma 2-like (BCL-XL) anti-apoptotic protein[156]. A role for DNA repair in overcoming DNA damage from reactive oxygen species, leading to loss of viability, has been documented for brain metastasis of breast cancer[164].

- The role of autophagy has been debated in cancer, but multiple reports link autophagy with increased metastatic colonization as an adaptive survival mechanism[165,166]. The autophagic response in colonization is negatively regulated by the NMYC downstream-regulated gene 1 (NDRG1) metastasis suppressor[167].

- Multiple genes involved in metastatic dissemination have been shown to have roles in metastatic colonization, using haematogenous metastasis assays. These include SRC[168], diverse proteases, transforming growth factor-β (TGFβ)[169,170], RHO family members[171], β-catenin[172] and cell adhesion molecules. Transcriptional programmes induced by SNAIL (also known as SNAI1)[173], inhibitor of DNA binding 1 (ID1)[174] and GATA binding protein 3 (GATA3)[175] are also operative in metastatic colonization.

- Non-coding RNAs such as microRNAs (miRNAs)[176–178] and long non-coding RNAs[179] regulate complex gene expression patterns to effect colonization.

- Pathways operative in generating tumour-initiating cells functionally promote metastatic colonization[180–182].

- Protein expression patterns are often distinct in metastases, including the downregulation of metastasis suppressor genes[183]. Metastasis suppressors inhibit many steps in colonization including tumour cell transcriptional programmes[184,185], survival after arrival in a distant organ[186,187], stress-induced autophagy[167], vasoconstriction[188] and cell cycle progression[189].

- Metastatic tumour cell phenotypes are plastic. Tumour cells may undergo an epithelial–mesenchymal transition (EMT) to invade, which is reversed (mesenchymal–epithelial transition, MET) in colonization. It has been reported that layered onto this plasticity is transdifferentiation of tumour cells to myofibroblasts in the microenvironment. The discovery of overarching cellular programmes controlling this plasticity may represent another therapeutic opportunity in metastasis.

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a Premetastatic niche

- CAIX
- G-CSF
- HIF1

VEGF

VEGF

Inflammatory cytokines

Altered metabolism

- Exosomes
- MIF
- miR-122

- VEGF
- TNF
- TGFβ

- S100A8
- S100A9
- SAA

Extravasation

- CCL2 • CCL15
- CCL9 • TGFβ

b ECM and fibroblasts

Proteases

Fibrosis   Proteases

c Innate immune cell recruitment

PPARγ

TLR

↓IFNγ   ↓TNF

CX3CL1

CCL2 and CCL3

Osteoclast progenitor

Bone metastasis

- FGF
- Hypoxia
- TGFβ

CXCL16   CCR4

Educated, pro-angiogenic bone marrow-derived cells

d Recruitment of T cells

- CCL17
- CCL22

↑PD1

- IL-1β
- IL-17
- G-CSF

↑IFNγ   ↑PDL1

e Angiogenesis

- Angiogenesis
- VEGF
- ANGPT

Vasculogenic mimicry

Distant metastatic site

Normal vasculature

Legend

Tumour-initiating cell	T_reg cell	Fibronectin	Neutrophil	CTL	Versican
Tumour cell	CD8+ T cell	Collagen	Macrophage	Exosome	Tenascin C
Hypoxic tumour cell	NK cell	S100A8 and S100A9 cytokines	Fibroblast	LOX	Hyaluronic acid
Invasive tumour cell	MDSC	Other cytokines	Myofibroblast	ROS	

Preclinical metastasis prevention experiments include anti-chemokine (C-C motif) ligand 2 (CCL2) for liver metastases[118] and anti-CXCL12 for prevention of non-small-cell lung cancer metastasis[119]. An antisense strategy for CCL17 silenced genes in T regulatory (T_{reg}) cells and blocked lung metastasis in a breast cancer model[116]. AMD3100 is a small-molecule inhibitor

of CXCR4, which has FDA approval for stem cell mobilization (FDA approval for plerixafor), and has preclinical antimetastatic activity in ovarian cancer[120]. This drug is in widespread clinical testing in haematological tumours; an early-stage trial in metastatic pancreatic, ovarian and colorectal cancers is currently recruiting[121].

An issue in the development of drugs that target chemokines is the existence of multiple family members with potentially overlapping functions. For instance, melanoma metastasis to the brain upregulated the expression of CXCL10, CCL4 and CCL17 in cerebrospinal fluid[122]. It will be of interest to determine how the immune functions of chemokine targeting interact with immune checkpoint therapy.

Lysyl oxidase. Lysyl oxidase (LOX) is a secreted enzyme, induced by hypoxia, that crosslinks collagens and elastin in the ECM. Its mechanism of action in metastatic colonization includes mobilization of bone marrow cells in the premetastatic niche and alterations in the ECM, which are thought to alter integrin engagement and survival signalling (FIG. 2a,b). An antibody to LOX, as well as β-aminopropionitrile (BAPN), a small-molecule inhibitor of the LOX family, prevented the formation of breast cancer metastases[123]. Interestingly, both agents stabilized metastatic burden when administered at progressively later times in an experimental metastasis assay.

LOX-like 2 (LOXL2), is a relative of LOX that binds the E47 transcription factor and alters the expression of fibronectin and cytokines in the lung to affect bone marrow recruitment[124]. An antibody to LOXL2, simtuzumab, was tested in a randomized Phase II trial in combination with chemotherapy in refractory metastatic colorectal cancer. Using a PFS end point, simtuzumab did not improve outcome beyond chemotherapy[125]. This trial suffers from issues that affected the SRC inhibitors: lack of an adjuvant trial to match preclinical data that demonstrated metastasis prevention, and reliance on synergy with chemotherapy. The LOXL family has also been associated with both suppression of tumorigenesis and stimulation of metastasis[126], and patient selection may be a key issue for this and other trials.

Exosomes. Exosomes are small, cell-derived vesicles that promote cell–cell communication. Besides their biological roles in metastasis, they may constitute a technological marvel. Exosomes have roles in the premetastatic niche, angiogenesis, immune function and tumour cell communication with the microenvironment (FIG. 2a,e). Exosome cargoes are heterogeneous, both metastasis suppressive[127–129] and metastasis accelerating[130–133]. Clearly, additional work is needed in the field to sort out the complexity of exosome cargoes as well as the conditions facilitating their release and uptake. Detection of exosomes or their cargo may provide a liquid biopsy of minimal residual disease to enable earlier interventions in trials.

Exosomes also hold translational importance as an engineering platform. Although the strategy is in its infancy, exogenous small interfering RNAs (siRNAs) have been engineered into exosomes and delivered *in vivo*[134], suggesting that metastasis-suppressive cargoes could

◀ Figure 2 | **Functional interactions between tumour cells and the metastatic microenvironment in colonization. a** | Premetastatic niche. Primary tumour cells upregulate vascular endothelial growth factor (VEGF), causing VEGF receptor-positive (VEGFR+) haematopoietic bone marrow cells (also called myeloid-derived suppressor cells (MDSCs)) to migrate to the lung, upregulating fibronectin deposition in the extracellular matrix (ECM) by resident fibroblasts and producing inflammatory cytokines. Disseminated tumour cells then home to these locations for preferential colonization[206,207]. A premetastatic niche is also formed by tumour secretion of VEGF, tumour necrosis factor (TNF) or transforming growth factor-β (TGFβ), stimulating lung tissue to produce S100A8 and S100A9 chemokines, which serve as chemoattractants for alveolar and peritoneal macrophages and tumour cells[208,209]. Primary tumour hypoxic conditions favour formation of a premetastatic niche by producing lysyl oxidase (LOX) to alter the microenvironment[210], carbonic anhydrase (CAIX) to mobilize MDSCs[211], and suppression of natural killer (NK) cell activation[212]. Exosomes produced by the primary tumour educate MDSCs and alter the premetastatic microenvironment ECM and metabolism directly[130,131,213]. **b** | ECM and fibroblasts. Colonizing tumour cells functionally interact with altered levels of hyaluronic acid, fibronectin, tenascin C and collagens in the ECM. The ECM is remodelled by various proteases produced by tumour cells and the activated microenvironment, with downstream effects on adhesion and tumour viability[123]. Integrins, receptors for ECM components, mediate many interactions between tumour cells and the altered ECM to effect colonization[58]. Fibroblasts in the microenvironment are activated by tumour cells or their secreted factors; comigration of primary tumour fibroblasts with tumour cells to metastatic sites also occurs in model systems[214]. Activated fibroblasts contribute to multiple aspects of colonization, including angiogenesis, inflammation, immunity and tumour growth potential. Fibrosis is an out-of-control activation of myofibroblasts to produce higher amounts of ECM. When fibrosis is induced by drugs or radiation treatment, experimental metastases are elevated[215]. **c** | Innate immunity. Bone marrow-derived myeloid cells, which are macrophage-like, are stimulated to migrate to sites of metastasis by TGFβ, which promotes metastatic colonization by diminishing arginase, reactive oxygen species (ROS) and interferon-γ (IFNγ) production, leading to decreased T cell-dependent antitumour immunity[198]. Other tumour-derived factors[118,216], ECM components[217] and hypoxia mobilize myeloid cells. Their activity is regulated by growth factors[218], toll-like receptor (TLR)[217] and peroxisome proliferator-activated receptor-γ (PPARγ)[219] signalling; a chemokine (C-X3-C motif) ligand 1 (CX3CL1) loop promotes their viability[115]. Myeloid cells also transdifferentiate into metastasis effector cells[220]. Macrophages from host tissue and circulation also facilitate colonization through chemokine cascades[114]. Reciprocal signalling between macrophages and tumour cells enhances the viability of both cell populations[115,221]. NK cells also join the site of colonization and provide innate immune functions. **d** | T cell-mediated immunity. Influx and activation of tumour infiltrating lymphocytes (TILs) is mediated by chemokines such as C-X-C motif chemokine ligand 16 (CXCL16)[117]. Programmed cell death protein 1 (PD1) immune checkpoint expression is increased on CD8+ TILs in the metastatic microenvironment[222]. In turn, CD8+ TILs secrete IFNγ to upregulate PD1 ligand 1 (PDL1) on metastatic tumour cells[223,224]. The PD1–PDL1 pathway inactivates the cytotoxic T-lymphocyte (CTL) effector arm. Tumour cells halt immune responses in several ways. They produce interleukins (ILs), leading to the production of granulocyte-colony stimulating factor (G-CSF), mobilization of neutrophils and inactivation of CTLs[225]. Tumour chemokine networks can recruit T regulatory (T_{reg}) cells, shutting down NK cell activity[226]. A balance of tumour zinc finger E-box binding homeobox 1 (ZEB1) and microRNA miR-200 regulates PDL1 expression[227]. **e** | Vascular system. Angiogenesis is stimulated by hypoxia. Endothelial cells proliferate, migrate and encircle to form capillaries. The process is facilitated by MDSCs, which are 'educated' by tumour-derived exosomes[131]. Other pathways such as angiopoietin (ANGPT) signalling, stabilize vessels[52]. Residual hypoxia stimulates tumour invasion[24,49–51]. Other sources of blood supply include co-option of the existing vasculature and vasculogenic mimicry, which is the formation of blood-conducting tubes by tumour cells[55–57,228]. CCL, chemokine (C-C motif) ligand; CCR, chemokine (C-C motif) receptor; MIF, macrophage migration inhibitory factor; SAA, serum amyloid A.

Transforming growth factor-β (TGFβ) is a secreted protein that controls the proliferation and differentiation of cells. It binds to a receptor complex that recruits and phosphorylates SMAD family proteins. The SMAD proteins enter the nucleus and act as transcription factors. Other SMADs and regulatory proteins can block the pathway[190].

Both small-molecule inhibitors of, and antibodies to, TGFβ have been developed, and they prevented metastasis in several model systems[191,192]. Side effects measured in long-term experiments seemed nominal. One of the important advantages of TGFβ as a metastatic colonization target is that it affects multiple pathways, including extracellular matrix (ECM) remodelling[193], tumour–microenvironment interactions[194–196], transcriptional programmes[197], immunity[198], angiogenesis[193,199] and tumour cell viability[196,199] (FIG. 2a,c).

A potential problem is the switch in TGFβ function from a tumour suppressor in normal cells to a metastasis stimulator in aggressive cancer cells. The switch mechanism is complex and incompletely understood in mice but involves distinct intracellular signalling[194,200,201] and tumour–microenvironment interactions that favour myeloid-derived suppressor cell (MDSC)-mediated inflammation[198]. To the extent that metastases in humans have 'switched' their signalling profile, which is incompletely known, it can be hypothesized that TGFβ targeting will be an effective antimetastatic colonization approach. A recent study identified a gene signature based on the eukaryotic translation initiation factor (eIF) family of transcription factors as distinguishing suppression versus promotion of tumour progression[202]; this and other pathways can be tested for prediction of patient responses to TGFβ inhibitors and, if positive, used to enroll patients.

Other issues in drug development for this pathway include the number of specific TGFβ family members that are functionally involved in metastatic progression, and rational combinations with standard of care therapy. Early-stage trials of drugs that target TGFβ in patients with advanced cancer are under way with end points of safety, survival, response rate, immune function and serum markers of progression. These end points do not yet test metastasis prevention, which is an end point used in preclinical studies of these drugs[203].

Adaptive immunity
Part of the immune system by which memory is acquired after an initial response to a specific antigen.

Natural killer (NK) cells
Lymphocytes that are cytotoxic for virally infected or tumour cells, without the need for major histocompatibility complex (MHC) and T cell receptor signalling.

Minimal residual disease
In leukaemia, a low level of tumour cells or their products in patients apparently treated successfully, detectable only with molecular markers.

Astrocytes
Star-shaped cells in the brain and spinal cord that maintain the blood–brain barrier, provide nutrients, maintain ion balance and assist in injury repair.

Microglia
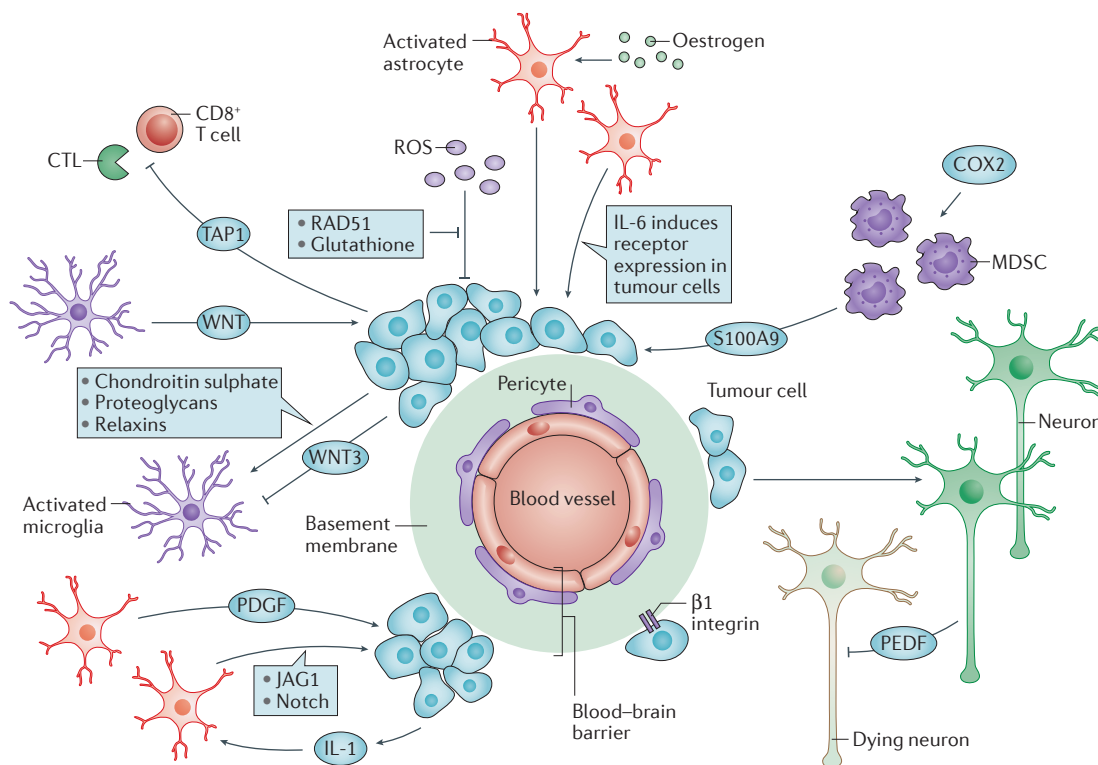Resident macrophage-like cells of the brain and spinal cord.

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eventually be delivered to target cells. Several exploratory clinical studies have opened to evaluate exosomes from cancer patients[135,136], potentially activate the immune system[137,138] and predict response to therapy[139]. In situations in which metastasis-promoting or immune-suppressive exosomes dominate, an exosome-removing trap using affinity plasmapheresis has been developed[140].

Site-specific metastases. Layered onto general pathways mediating metastatic colonization are functional interactions between tumour cells and aspects of the microenvironment that are either unique or selective. As an example, the vicious cycle of osteoclastic (bone-degrading) bone metastases involves RANKL, which has been successfully targeted by denosumab (TABLE 1). The good news is that many additional factors functionally interact to regulate bone metastasis formation and stand as prospective therapeutic targets to further improve patient outcomes. These include adhesive events (metadherin (MTDH; also known as LYRIC))[141], interleukin-11 (IL-11), hypoxia and chemokines. A pathway for delayed formation of osteolytic bone metastases centres on tumour cell SRC providing survival signalling against the apoptotic signals induced by CXCL12 and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) in the bone microenvironment[142].

A series of tumour–microenvironment functional interactions is also emerging for brain metastases, which are common in lung and breast cancers, and in melanoma[143] (FIG. 3). Some of these pathways overlap with those involved in metastatic colonization in other tissues, whereas other pathways are unique to the site. Reciprocal interactions have been functionally demonstrated between tumour cells and activated astrocytes and microglia that are recruited as a neuroinflammatory response to the presence of tumour cells[144]. Translational development of these metastasis pathways will also have to surmount the partially intact blood–brain barrier, making drug delivery inefficient[145].

Treating established metastases
Shrinking an established metastasis represents a higher bar than preventing the formation of an overt lesion. Drugs must kill tumour cells rather than just being cytostatic. Overt metastases contain millions of tumour cells. They often have a tortuous vasculature and elevated hydrostatic pressure, causing poor drug penetration. Other than the limited efficacy of cytotoxic drugs to produce responses and, at best, marginally enhance survival, what approaches hold promise?

Two general approaches are under development for treating established metastases. BOX 3 details the programmed cell death protein 1 (PD1; also known as PDCD1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) immune checkpoint inhibitor approaches. In general, these inhibitors are producing clinical responses and long-term PFS improvements in a subpopulation of patients with metastatic melanoma, lung cancer or renal cancer[146–149]. These cancers rank high on a graph of tumour neoantigen levels[90], suggesting that other, less antigenic, cancer types may not be immediately as responsive.

The second approach to shrinking established metastases is cytotoxic. Use of α-particle-emitting radionuclides, alone or linked to monoclonal antibodies, constitutes an avenue of some promise. These radionuclides offer the advantages of high energy to kill tumour cells, and low penetration, which minimizes damage to surrounding normal tissue. In a Phase III trial, radium-223 increased the survival of patients with metastatic prostate cancer[150]. Most of the other α-emitting radionuclide approaches attach the radionuclide to a monoclonal antibody that binds tumour tissue to a far greater extent than normal tissue, so that its applicability is limited only by the abundance and specificity of the antigens. Preclinical experiments in which FDA-approved monoclonal antibodies were stably linked to α-emitters demonstrated regressions in metastatic peritoneal cancer[151]. Similarly, addition of toxic drugs to an anti-HER2 monoclonal antibody enhanced response rates in breast cancer metastases[152].

With this abundance of worthwhile leads for preventing and treating metastases, momentum has built for translational progress. The next steps to consider are establishment of adequate preclinical credentials and clinical testing of potential metastasis-directed therapies.

Formulae for greater success
Preclinical experiments. Mouse experimentation for preclinical therapeutic development has been justifiably criticized for poor reproducibility. Many types of animal model exist, including xenografts, syngeneic

Figure 3 | **A wealth of mechanistic translational targets in metastasis to the brain.** In addition to the basic pathways of colonization, bidirectional interactions between tumour cells and the microenvironments of specific tissues foster metastatic colonization. In the brain, tumour cells breach the blood–brain barrier to extravasate using tissue-nonspecific and tissue-specific adhesion molecules and proteases[228,229]. Tumour cells then adhere to the vascular basement membrane via β1 integrins[230]. Activated astrocytes congregate around the developing metastasis and are stimulated by tumour-derived cytokines[231]. The astrocytes produce growth factors that are stimulatory for the tumour cells[231,232], and elevate tumour cell expression of receptors for cytokines in the microenvironment[233]. Systemic hormones such as oestrogen can further activate astrocytes to stimulate colonization[234]. Activated microglia, resident macrophage-like cells, also surround a developing metastasis. Their activation is determined by a balance of tumour cell stimulatory and inhibitory factors[235–238]. In turn, they activate tumour cells via the WNT pathway. The brain is infiltrated by myeloid-derived suppressor cells (MDSCs) in a premetastatic phase[239], fuelled by cyclooxygenase 2 (COX2) and T cells. Tumours shut down T cell responses using ATP binding cassette transporter 1 (TAP1; also known as ABCB2)[240]. The brain microenvironment mounts a protective reaction by the secretion of reactive oxygen species (ROS)[241]. Overexpression of DNA double strand break repair genes by tumour cells attenuates the ROS-induced DNA damage[164] and overexpression of serpins inactivates death signals from the microenvironment[242]. Neuronal cell death is a consequence of metastasis formation, but is attenuated by tumour-derived pigment-epithelial-derived factor (PEDF)[243]. It is likely that many of the mediators of brain metastatic colonization are active in other anatomical locations as well, via a different set of host microenvironmental cells, suggesting that any therapeutics that are developed may be more broadly applicable. CTL, cytotoxic T lymphocyte; IL-6, interleukin 6; JAG1, Jagged 1; PDGF, platelet-derived growth factor.

Neuroinflammatory response
Inflammation of the central nervous system characterized by activation of endothelial and glial cells, cytokines and oedema.

α-particle
A particle for radiation therapy consisting of a helium nucleus with high energy and low penetrance.

Xenografts
In cancer, cells or tissues transplanted from one species to another, often human cancer cells into immunodeficient mice.

Genetically engineered mouse (GEM) models
Mouse models in which the genome has been altered, including transgenes and targeted mutations (knockouts or knockins).

Patient-derived xenografts
(PDXs). Patient tumour tissues implanted directly into immunodeficient mice.

models, genetically engineered mouse (GEM) models and patient-derived xenografts (PDXs); each has strengths and weaknesses[153]. Traditional xenograft experiments involve injection of tumour cells subcutaneously or orthotopically into the tissue of origin. Mice bearing xenograft tumours are dosed with an investigational compound and the size of primary tumour growth is measured over time. Retardation of primary tumour growth is considered a success, whereas in the clinic, tumours growing at different rates are still growing and are considered progressive disease, potentially with increased patient PFS. In primary tumour xenograft models, the tumour cells have not interacted with the metastatic microenvironments at all, and this preclinical experimental design would only be relevant for targeting initial oncogenic trunk mutations and any effects they may have on metastasis.

Metastasis preclinical models are absolutely necessary for the development of metastasis-related therapeutics. Orthotopic injection of tumour cells to form a primary tumour and then seed metastases (spontaneous metastasis) is the gold standard. The full metastatic process is modelled in this manner. Similar experiments using PDXs eliminate the potential biases by culture of cell lines on plastic, and have been reported to recapitulate the sites of metastasis and response to therapy observed in the patients from which they are derived[154]. Some GEM models produce metastases. Weaknesses of spontaneous metastasis models include a paucity of metastases, usually producing 'yes or no' animal data, rendering small but potentially significant differences hard to validate, and requiring relatively long times for metastasis development.

Box 3 | **Immune therapy approaches for established metastatic disease**

Most translational metastasis research focuses on prevention, that is, mice are treated early and continuously with an investigational agent. Established metastases are often treated with radiation therapy and chemotherapy, but additional, efficacious, less toxic, avenues are needed. Two immune checkpoints have begun to turn the tide on immune therapy for patients with established metastatic disease.

- Adaptive responses to tumours involve antigen–major histocompatibility complex (MHC) protein binding to the T cell receptor. This is coordinated with a co-stimulatory interaction of the surface glycoprotein CD28 that is expressed on the T cell with a B7 receptor on the antigen-presenting cell. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) outcompetes CD28 for binding to B7 to reduce immune activation. Ipilimumab, a monoclonal antibody that blocks CTLA4, preclinically enhanced the effector T cell subpopulation while downregulating a suppressor T cell subpopulation[146,149]. Clinically, ipilimumab extended overall survival (OS) in resistant metastatic melanoma, as monotherapy or in combination with a vaccine, leading to US Food and Drug Administration (FDA) approval (FDA approval for ipilimumab). Hallmarks of its activity were an initial flare in lesion size before gradual shrinkage and long-term responses in the metastases of a minority of patients.

- Programmed cell death protein 1 (PD1, which is encoded by *PDCD1*) is a member of the CD28 co-stimulatory receptor family found on activated T cells, B cells and myeloid cells. Its upregulation on T cells after chronic antigen presentation is a marker of T cell exhaustion. PD1 ligand 1 (PDL1), is expressed by many tumour cells to dampen immune responses. In initial preclinical experiments animals were treated with PD1 antibodies or tumour cells were injected into *Pdcd1*−/− mice, which stabilized disease, both as monotherapy and in combination with other immunotherapy[147,148]. Both pembrolizumab and nivolumab, monoclonal antibodies that block PD1, show responses and long-term progression-free survival (PFS) in a proportion of patients with metastatic lung cancer or melanoma (FDA approval for pembrolizumab and nivolumab). Interestingly, PD-1 blockade was also highly effective in mismatch repair-deficient tumours, for instance, a subset of colorectal carcinomas[204].

- The combination of anti-PD1 and anti-CTLA4 therapy was superior to either therapy alone in untreated metastatic melanoma[205].

Injection of tumour cells directly into the circulation can mimic aspects of metastatic colonization (experimental metastasis). Tumour cells are injected into the tail vein, portal vein or left cardiac ventricle to colonize the lungs, liver, and bone and brain, respectively. Tumour cells can also be injected into a body cavity, such as the peritoneum. In experimental metastasis models, a bolus of tumour cells is injected rather than a constant stream of disseminating cells from a primary tumour as would happen in the natural situation, and the pre-metastatic niche has not preformed. However, for drug development, these assays measure most of the process of metastatic colonization, and provide good quantification in a reasonable time frame.

Beyond these generalities several considerations are important. FIGURE 4 shows potential designs of preclinical models for the development of metastatic therapies. Most commonly, the designs in FIG. 4b,d are reported, using either spontaneous or experimental metastasis assays and delivering the investigational compound from the beginning of the experiment onwards. However, the adjuvant setting is best modelled by administration of the investigational compound after primary tumour removal (FIG. 4c) or, potentially, treatment delayed until after tumour cells have extravasated (FIG. 4d).

Additional parameters can be considered using osteolytic bone metastasis (TABLE 1) as an example. Bone metastases occur predominantly in ER+ breast cancer and this

Micrometastases
Metastatic lesions that are too small for conventional detection.

subtype of cell line should be used in the preclinical model. ER+ breast cancer cells can form bone metastases following left cardiac ventricle injection[155]. The investigational compound can be started after tumour cell injection in a prevention scenario, or after the development of lesions in a metastatic setting. Incorporation of SOC therapy is an often overlooked but important variable. The SOC could include an anti-hormonal treatment and denosumab. The relationship of SOC and investigational agent dosing must be decided — will the investigational agent be dosed with denosumab, or following progression on denosumab? Most of the proposed osteolytic bone metastasis investigational agents have not demonstrated interaction with, or superiority to, denosumab, which constitutes incomplete preclinical validation.

Traditional drug development parameters also matter. Was a clinically achievable dose and schedule of compounds used? Was the route of administration comparable to what will be used in the clinic, typically oral or intravenous? Did the compound hit its target? Can these data be used to identify and test biomarkers or pharmacodynamic markers for prediction of clinical responses?

For outcomes, imaging must be used in conjunction with histopathology. Has the model just produced micrometastases? These lesions could persist without complications in the human, or could progressively grow, so their importance is uncertain. How are micrometastases and macrometastases delineated? Data from more than one metastasis model system, and often more than one target organ are needed; examples exist in which metastasis rates vary in different organs[156]. In addition to metastasis counts, survival end points can be used.

The next generation of models will evaluate clonal mutational dynamics, in both treatment-naive and treatment-resistant settings[157]. Deep sequencing will also be valuable in identifying tumour neoantigens as candidates and biomarkers of immunotherapy[90]. More models must be developed to reflect the heterogeneity of human disease. These improved models must be tied to expanded data on the transcriptional and genomic diversity in human metastases.

Embracing combination therapies. Given the extraordinary genomic and phenotypic instability of metastatic tumour cells, it is almost inconceivable that a single metastasis-directed monotherapy will cure the majority of patients. Human immunodeficiency virus (HIV) is another deadly therapeutic target that, like metastatic cancer, easily mutates. Initial efforts with monotherapies produced some responses but they lacked durability. Recognizing that a dire public health emergency required concerted efforts, two and eventually three drugs produced by different companies were combined into an effective, durable therapy that minimized the development of resistance[158]. Other important aspects of this effort that are salient to metastasis research include combining distinct classes of drugs, and having a test for minimal residual disease to monitor efficacy. Along with the success of the original 3-drug combination, 25 drugs in 6 classes have received FDA approval, providing back-up regimens.
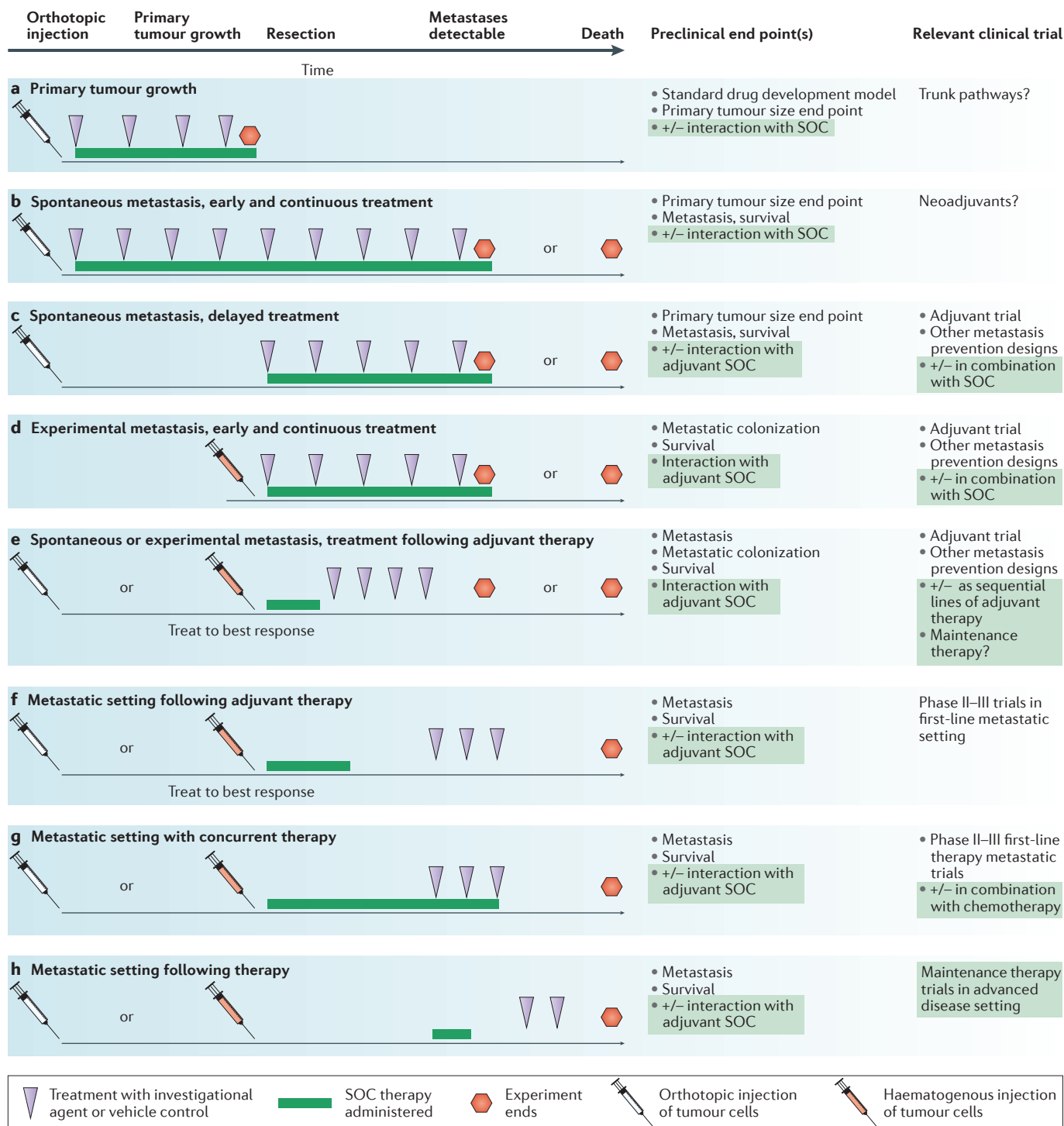
Figure 4 | **Meaningful incorporation of preclinical metastasis models into drug development.** Potential experimental designs for an antimetastatic investigational agent are mapped along a timeline. Tumour cell injection can either be orthotopic, into the tissue of origin (white needles) or haematogenous for experimental metastasis (red needles). The investigational agent (purple arrowheads) can be delivered for all or part of the assay; optimally it should reflect the oral or intravenous dosing to be used in the clinic. Standard of care (SOC) therapy (green line), at a clinically achievable dose, can be added before, concurrent with or after the investigational agent and can use agents approved in the adjuvant or metastatic setting. **a** | Standard drug development uses primary tumour growth as an end point, which completely neglects the metastatic process. **b–e** | Model systems with different degrees of applicability to adjuvant setting trials preventing metastatic colonization. **f–h** | Model systems with different applicabilities to metastatic setting trials. End points include the number and size of metastases by histology and imaging, pharmacodynamic markers of drug activity or tumour biology, and survival.

Clinical trials for metastasis-directed therapies. It is hypothesized that interruption of metastatic pathways will significantly augment the benefits of current cancer therapies. Because these pathways are functional in most cancer types, drug development and successful clinical testing offers the possibility of a blockbuster drug that is applicable to many patients[159]. Most preclinical metastasis therapy experiments point to a significant delay in the development of metastases rather than a shrinkage of established metastatic lesions. This end point should be beneficial to the clinical prevention of an initial metastasis, or the prevention of additional metastases in patients with limited, treated, metastatic disease, in adjuvant setting trials.

Many antimetastatic therapies under development interrupt colonization pathways rather than kill a proliferating tumour cell. They are cytostatic, not cytotoxic. Such agents will shrink an established lesion only if they coincidentally synergize with chemotherapy or radiation therapy. These facts lead to the disturbing conclusion that antimetastatic therapies will not produce traditional responses (complete response (CR) or partial response (PR)) in metastatic setting trials — stable disease would be the best expected result of a cytostatic agent. It is likely that many compounds with metastasis-preventive activity have failed in traditional clinical trials and are lost.

Designs that are germane to the preclinical end point of prevention or delay of metastasis include trials in the adjuvant setting. Adjuvant trials typically deliver systemic therapy following initial surgery to patients with colon, lung, pancreatic, breast, prostate and other cancers. The hypothesis is that tumour cells have already escaped the primary mass and are residing in distant locations as occult tumour cells or micrometastases and so adjuvant therapy should prevent their outgrowth. Primary end points include PFS and OS. There are substantial problems implementing adjuvant trials for potential metastasis-preventive therapies: adjuvant trials typically require large patient populations, take a relatively long time to mature and incur high costs. Thus they are considered only when there is a wealth of positive earlier clinical data. This is a problem if a cytostatic metastasis-preventive therapy has not produced responses in traditional Phase II trials of patients with metastatic disease.

Several alternative trial designs could measure metastasis prevention. Smaller trials using super-high-risk patients such as those with multiple positive lymph nodes could be useful for a signal of antimetastatic activity[160].

Metastatic disease would develop more rapidly and in a higher percentage of patients, minimizing the time needed and the size of the cohort.

For some cancers initial metastatic lesions are successfully treated, but the patient remains at an unacceptably high risk for the development of additional metastases. These patients can be randomized to a metastasis-preventive agent or placebo in a randomized Phase II secondary metastasis prevention trial. Examples include post-curative surgery for liver metastases[161], and post-neurosurgery or stereotactic radiotherapy for brain metastases[160]. The primary end point would be time to a new metastasis. Alternative end points such as circulating tumour cells or circulating tumour DNA can be investigated. If eventually validated, it is possible that these easily accessed biopsy methods could serve as earlier end points to hasten clinical trials.

In lung cancer, advanced disease is treated with chemotherapy, resulting in clinical benefit that is often short term in duration. Phase III randomized maintenance therapy trials have been extensively conducted. Patients with advanced cancer received standard initial chemotherapy and were then randomized to a long course of investigational therapy (chemotherapy or truncal mutation inhibitor therapy) or placebo until progression or unacceptable toxicity occurred; end points were PFS or OS. Such designs could be adapted to include a metastasis-preventive agent, to halt further colonization or to induce dormancy. Similar trial designs of long-term treatment with a low dose of drug have been proposed to induce metastatic dormancy in genitourinary cancers, called metronomic therapy[3].

Conclusion

Interruption of metastasis pathways holds preclinical and clinical promise for cancer patients with, or at risk of, metastatic disease. Multiple agents have been demonstrated to prevent or shrink metastases and serve as targets for therapeutic development. Lessons learned from HIV therapeutic development may prove useful, that is, to combine multiple classes of therapy, treat early and have multiple secondary options.

It is the responsibility of researchers to provide preclinical model data that adequately validate a potential antimetastasis therapeutic agent. Furthermore, there should be a responsibility to prioritize and fund grants to perform this work. Given the hesitancy of the pharmaceutical industry, academic and government organizations will need to fund alternative metastasis prevention trials until a sign of success emerges.

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Competing interests statement
The authors declare competing interests: see Web version for details.